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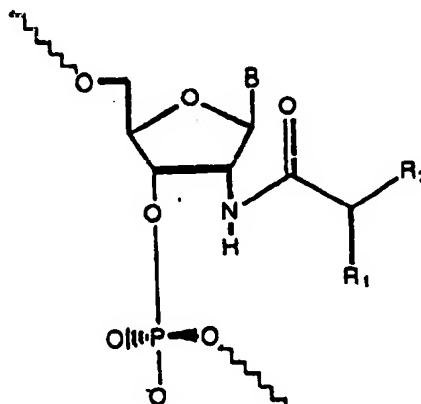
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(54) Title: 2'-AMIDO AND 2'-PEPTIDO MODIFIED OLIGONUCLEOTIDES

(57) Abstract

Oligonucleotide comprising a nucleotide base having formula (I) wherein B is a nucleotide base or hydrogen; R_1 and R_2 indepently are selected from the group consisting of hydrogen, an alkyl group containing between 2 and 10 carbon atoms inclusive, an amine, an amino acid, and a peptide containing between 2 and 5 amino acids inclusive; and the zigzag lines are independently hydrogen or a bond.

(I)

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DESCRIPTION

2'-AMIDO AND 2'-PEPTIDO MODIFIED OLIGONUCLEOTIDES

Background of the Invention

This invention relates to 2'- modifications of
oligonucleotides.

Usman et al., "Nucleozymes", International
5 Application No. PCT/US93/00833, describe modification of
the 2'-hydroxyl group of RNA to produce modified
nucleotides. Such nucleotides are termed nucleic acid
analogues, and may have a "good coordinating ligand" with
divalent metal ions, e.g., a halogen, or amine group.
10 Acyclic analogues are also proposed.

Eckstein, International Application No.
PCT/EP91/01811 (WO 92/0/065), describes 2'-hydroxyl
modifications of RNA having the following substitutions in
place of the hydroxyl group: halo, sulfhydryl, azido,
15 amino, monosubstituted amino and disubstituted amino.

Summary of the Invention

This invention relates to replacement of the 2'-
hydroxyl group of a ribonucleotide moiety with a 2'-amido
or 2'-peptido moiety. Generally, such a nucleotide has
20 the general structure shown in Formula I below:

Formula I

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The base (B) is any one of the standard bases or is a modified nucleotide base known to those in the art, or can be a hydrogen group. In addition, either R_1 or R_2 is H or an alkyl, alkene or alkyne group containing between 2 and
5   10 carbon atoms, or hydrogen, an amine (primary, secondary or tertiary, <u>e.g.</u>, R_3NR_4 where each R_3 and R_4 independently is hydrogen or an alkyl, alkene or alkyne having between 2 and 10 carbon atoms, or is a residue of an amino acid, <u>i.e.</u>, an amide), an alkyl group, or an amino acid (D or L
10   forms) or peptide containing between 2 and 5 amino acids. The zigzag lines represent hydrogen, or a bond to another base or other chemical moiety known in the art. Preferably, one of R_1 and R_2 is an H, and the other is an amino acid or peptide.
15   Applicant has recognized that RNA can assume a much more complex structural form than DNA because of the presence of the 2'-hydroxyl group in RNA. This group is able to provide additional hydrogen bonding with other hydrogen donors, acceptors and metal ions within the RNA
20   molecule. Applicant now provides molecules which have a modified amine group at the 2' position, such that significantly more complex structures can be formed by the modified oligonucleotide. Such modification with a 2'-amido or peptido group leads to expansion and enrichment
25   of the side-chain hydrogen bonding network. The amide and peptide moieties are responsible for complex structural formation of the oligonucleotide and can form strong complexes with other bases, and interfere with standard base pairing interactions. Such interference will allow
30   the formation of a complex nucleic acid and protein conglomerate.
         Oligonucleotides of this invention are significantly more stable than existing oligonucleotides and can potentially form biologically active bioconjugates
35   not previously possible for oligonucleotides. They may also be used for *in vitro* selection of unique aptamers, that is, randomly generated oligonucleotides which can be

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folded into an effective ligand for a target protein, nucleic acid or polysaccharide.

Thus, in a first aspect, the invention features an oligonucleotide containing the modified base shown in
5   Formula I, above.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

10   Oligonucleotides of this invention are described generally above, and the structure is shown in Formula I, where such modifications to the 2'-hydroxyl group can be made in one or more positions of an RNA or DNA molecule. Preferably, the oligonucleotide is single-stranded and has
15   between 10 and 50 bases of which one or more may be modified as shown, preferably, between 1 and 10 are modified. Such oligonucleotides may include those having enzymatic activity, i.e., ribozymes, which are modified in the 2'- position of the sugar moiety as shown in Formula
20   I to provide stability to that enzymatic activity without significant alteration of the activity.

Oligonucleotides of the present invention can be readily synthesized using carbamate protecting groups, such as F-moc, in the peptide moieties and deprotected
25   under mild basic conditions. Such nucleotides can then be incorporated by standard solid phase synthesis using nucleoside phosphoramidite or H-phosphonate intermediates.

Use

The above nucleotides are particularly useful in
30   ribozymes. Ribozymes are RNA molecules having an enzymatic activity which is able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence specific manner. Such enzymatic RNA molecules can be targeted to virtually any RNA transcript, and efficient
35   cleavage achieved in vitro. Kim et al., 84 Proc. Natl. Acad. Sci. USA 8788, 1987; Haseloff and Gerlach, 334

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Nature 585, 1988; Cech, 260 _JAMA_ 3030, 1988; and Jefferies
et al., 17 _Nucleic Acids Research_ 1371, 1989.

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Ribozymes act by first binding to a target RNA.
Such binding occurs through the target RNA binding portion
of a ribozyme which is held in close proximity to an
enzymatic portion of the RNA which acts to cleave the
target RNA. Thus, the ribozyme first recognizes and then
binds a target RNA through complementary base-pairing, and
once bound to the correct site, acts enzymatically to cut
the target RNA. Strategic cleavage of such a target RNA
will destroy its ability to direct synthesis of an encoded
protein. After a ribozyme has bound and cleaved its RNA
target it is released from that RNA to search for another
target and can repeatedly bind and cleave new targets.

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The enzymatic nature of a ribozyme is
advantageous over other technologies, such as antisense
technology (where a nucleic acid molecule simply binds to
a nucleic acid target to block its translation) since the
effective concentration of ribozyme necessary to effect a
therapeutic treatment is lower than that of an antisense
oligonucleotide. This advantage reflects the ability of
the ribozyme to act enzymatically. Thus, a single
ribozyme molecule is able to cleave many molecules of
target RNA. In addition, the ribozyme is a highly
specific inhibitor, with the specificity of inhibition
depending not only on the base pairing mechanism of
binding, but also on the mechanism by which the molecule
inhibits the expression of the RNA to which it binds.
That is, the inhibition is caused by cleavage of the RNA
target and so specificity is defined as the ratio of the
rate of cleavage of the targeted RNA over the rate of
cleavage of non-targeted RNA. This cleavage mechanism is
dependent upon factors additional to those involved in
base pairing. Thus, it is thought that the specificity of
action of a ribozyme is greater than that of antisense
oligonucleotide binding the same RNA site.

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By "enzymatic RNA molecule" it is meant an RNA molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave

5   RNA in that target. That is, the enzymatic RNA molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. This complementarity functions to allow sufficient hybridization of the enzymatic RNA molecule to the target RNA to allow the

10   cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. By "equivalent" RNA to picornavirus is meant to include those naturally occurring RNA molecules associated with viral caused diseases in

15   various animals, including humans, and other primates. These viral RNAs have similar structures and equivalent genes to each other.

In preferred embodiments the enzymatic RNA molecule is formed in a hammerhead motif, but may also be

20   formed in the motif of a hairpin, hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence). Examples of such hammerhead motifs are described by Rossi et al., 8 Aids Research and Human Retroviruses 183, 1992; of hairpin motifs by Hampel et

25   al., "RNA Catalyst for Cleaving Specific RNA Sequences," filed September 20, 1989, which is a continuation-in-part of U.S. Serial No. 07/247,100 filed September 20, 1988, Hampel and Tritz, 28 Biochemistry 4929, 1989 and Hampel et al., 18 Nucleic Acids Research 299, 1990; and an example

30   of the hepatitis delta virus motif is described by Perrotta and Been, 31 Biochemistry 16, 1992; of the RNaseP motif by Guerrier-Takada et al., 35 Cell 849, 1983; and of the group I intron by Cech et al., U.S. Patent 4,987,071. These specific motifs are not limiting in the invention

35   and those skilled in the art will recognize that all that is important in an enzymatic RNA molecule of this invention is that it has a specific substrate binding site

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which is complementary to one or more of the target gene
RNA regions, and that it have nucleotide sequences within
or surrounding that substrate binding site which impart an
RNA cleaving activity to the molecule.

5 Synthesis of Ribozymes

    Ribozymes useful in this invention can be
produced by chemical synthesis. Chemical synthesis of RNA
is similar to that for DNA synthesis. The additional 2'-
OH group in RNA, however, requires a different protecting
10 group   strategy   to   deal   with   selective   3'-5'
internucleotide   bond   formation,   and   with   RNA
susceptibility to degradation in the presence of bases.
The recently developed method of RNA synthesis utilizing
the t-butyldimethylsilyl group for the protection of the
15 2'-hydroxyl is the most reliable method for synthesis of
ribozymes.   The method reproducibly yields RNA with the
correct 3'-5' internucleotide linkages, with average
coupling yields in excess of 99%, and requires only a two-
step deprotection of the polymer.

20   A method based upon H-phosphonate chemistry
gives a relatively lower coupling efficiency than a method
based upon phosphoramidite chemistry. This is a problem
for synthesis of DNA as well. A promising approach to
scale-up of automatic oligonucleotide synthesis has been
25 described recently for the H-phosphonates. A combination
of a proper coupling time and additional capping of
"failure" sequences gave high yields in the synthesis of
oligodeoxynucleotides in scales in the range of 14 μmoles
with as little as 2 equivalents of a monomer in the
30 coupling step.   Another alternative approach is to use
soluble polymeric supports (e.g., polyethylene glycols),
instead of the conventional solid supports. This method
can yield short oligonucleotides in hundred milligram
quantities per batch utilizing about 3 equivalents of a
35 monomer in a coupling step.

          Various modifications to ribozyme structure can
be made to enhance the utility of ribozymes.   Such

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modifications will enhance shelf-life, half-life *in vitro*,
stability, and ease of introduction of such ribozymes to
the target site, <u>e.g.</u>, to enhance penetration of cellular
membranes, and confer the ability to recognize and bind to
5   targeted cells.

          Exogenous delivery of ribozymes benefits from
chemical modification of the backbone, <u>e.g.</u>, by the
overall negative charge of the ribozyme molecule being
reduced to facilitate diffusion across the cell membrane.
10   The present strategies for reducing the oligonucleotide
charge include: modification of internucleotide linkages
by ethylphosphonates, use of phosphoramidites, linking
oligonucleotides to positively charged molecules, and
creating complex packages composed of oligonucleotides,
15   lipids and specific receptors or effectors for targeted
cells. Examples of such modifications include sulfur-
containing ribozymes containing phosphorothioates and
phosphorodithioates as internucleotide linkages in RNA.
Synthesis of such sulfur-modified ribozymes is achieved by
20   use of the sulfur-transfer reagent, ^{3}H-1,2-benzenedithiol-
3-one 1,1-dioxide. Ribozymes may also contain ribose
modified ribonucleotides as described herein. Ribozymes
can also be either electrostatically or covalently
attached to polymeric cations for the purpose of reducing
25   charge. The polymer can be attached to the ribozyme by
simply converting the 3'-end to a ribonucleoside
dialdehyde which is obtained by a periodate cleavage of
the terminal 2',3'-cis diol system. Depending on the
specific requirements for delivery systems, other possible
30   modifications may include different linker arms containing
carboxyl, amino or thiol functionalities. Yet further
examples include use of methylphosphonates and 2'-O-
methylribose and 5' or 3' capping or blocking with m_7GpppG
or $m_3^{2,2,7}$GpppG.
35   For example, a kinased ribozyme is contacted
with guanosine triphosphate and guanyltransferase to add
a m^3G cap to the ribozyme. After such synthesis, the

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ribozyme can be gel purified using standard procedure. To ensure that the ribozyme has the desired activity, it may be tested with and without the 5' cap using standard procedures to assay both its enzymatic activity and its
5 stability.

Synthetic ribozymes, including those containing various modifiers, can be purified by high pressure liquid chromatography (HPLC). Other liquid chromatography techniques, employing reverse phase columns and anion
10 exchangers on silica and polymeric supports may also be used.

There follows an example of the synthesis of one ribozyme. A solid phase phosphoramidite chemistry is employed. Monomers used are 2'-tert-butyl-dimethylsilyl
15 cyanoethylphosphoramidites of uridine, N-benzoyl-cytosine, N-phenoxyacetyl adenosine and guanosine (Glen Research, Sterling, VA). Solid phase synthesis is carried out on either an ABI 394 or 380B DNA/RNA synthesizer using the standard protocol provided with each machine. The only
20 exception is that the coupling step is increased from 10 to 12 minutes. The phosphoramidite concentration is 0.1 M. Synthesis is done on a 1 μmole scale using a 1 μmole RNA reaction column (Glen Research). The average coupling efficiencies are between 97% and 98% for the 394
25 model, and between 97% and 99% for the 380B model, as determined by a calorimetric measurement of the released trityl cation.

Blocked ribozymes are cleaved from the solid support (e.g., CPG), and the bases and diphosphoester
30 moiety deprotected in a sterile vial by dry ethanolic ammonia (2 mL) at 55°C for 16 hours. The reaction mixture is cooled on dry ice. Later, the cold liquid is transferred into a sterile screw cap vial and lyophilized. These conditions are suitable for removal of carbamate
35 blocking groups in the 2' modifications shown in Formula I, and the amido and peptido linkages remain intact.

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To remove the 2'-tert-butyl-dimethylsilyl groups
from the ribozyme, the residue is suspended in 1 M tetra-
n-butylammonium fluoride in dry THF (TBAF), using a 20-
fold excess of the reagent for every silyl group, for 16
5　hours at ambient temperature (about 15-25°C). The
reaction is quenched by adding an equal volume of sterile
1 M triethylamine acetate, pH 6.5. The sample is cooled
and concentrated on a SpeedVac to half the initial volume.

The ribozymes are purified in two steps by HPLC
10　on a C4 300 Å 5 mm DeltaPak column in an acetonitrile
gradient.

The first step, or "trityl on" step, is a
separation of 5'-DMT-protected ribozyme(s) from failure
sequences lacking a 5'-DMT group. Solvents used for this
15　step are: A (0.1 M triethylammonium acetate, pH 6.8) and
B (acetonitrile). The elution profile is: 20% B for 10
minutes, followed by a linear gradient of 20% B to 50% B
over 50 minutes, 50% B for 10 minutes, a linear gradient
of 50% B to 100% B over 10 minutes, and a linear gradient
20　of 100% B to 0% B over 10 minutes.

The second step is a purification of a
completely deblocked ribozyme by a treatment of 2%
trifluoroacetic acid on a C4 300 Å 5 mm DeltaPak column in
an acetonitrile gradient. Solvents used for this second
25　step are: A (0.1 M Triethylammonium acetate, pH 6.8) and
B (80% acetonitrile, 0.1 M triethylammonium acetate, pH
6.8). The elution profile is: 5% B for 5 minutes, a
linear gradient of 5% B to 15% B over 60 minutes, 15% B
for 10 minutes, and a linear gradient of 15% B to 0% B
30　over 10 minutes.

The fraction containing ribozyme is cooled and
lyophilized on a SpeedVac. Solid residue is dissolved in
a minimum amount of ethanol and sodium perchlorate in
acetone. The ribozyme is collected by centrifugation,
35　washed three times with acetone, and lyophilized.

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Administration of Ribozyme

Selected ribozymes can be administered prophylactically, or to diseased patients, e.g., by exogenous delivery of the ribozyme to an infected tissue

5 by means of an appropriate delivery vehicle, e.g., a liposome, a controlled release vehicle, by use of iontophoresis, electroporation or ion paired molecules, or covalently attached adducts, and other pharmacologically approved methods of delivery. Routes of administration

10 include intramuscular, aerosol, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal. Expression vectors for immunization with ribozymes and/or delivery of ribozymes are also suitable.

The specific delivery route of any selected

15 ribozyme will depend on the use of the ribozyme. Generally, a specific delivery program for each ribozyme will focus on naked ribozyme uptake with regard to intracellular localization, followed by demonstration of efficacy. Alternatively, delivery to these same cells in

20 an organ or tissue of an animal can be pursued. Uptake studies will include uptake assays to evaluate cellular oligonucleotide uptake, regardless of the delivery vehicle or strategy. Such assays will also determine the intracellular localization of the ribozyme following

25 uptake, ultimately establishing the requirements for maintenance of steady-state concentrations within the cellular compartment containing the target sequence (nucleus and/or cytoplasm). Efficacy and cytotoxicity can then be tested. Toxicity will not only include cell

30 viability but also cell function.

Some methods of delivery that may be used include:

a.   encapsulation in liposomes,

b.   transduction by retroviral vectors,

35   c.   conjugation with cholesterol,

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    d.   localization to nuclear compartment utilizing antigen binding site found on most snRNAs,

    e.   neutralization of charge of ribozyme by using nucleotide derivatives, and

    f.   use of blood stem cells to distribute ribozymes throughout the body.

At least three types of delivery strategies are useful in the present invention, including: ribozyme modifications, particle carrier drug delivery vehicles, and retroviral expression vectors. Unmodified ribozymes, like most small molecules, are taken up by cells, albeit slowly. To enhance cellular uptake, the ribozyme may be modified essentially at random, in ways which reduces its charge but maintains specific functional groups. This results in a molecule which is able to diffuse across the cell membrane, thus removing the permeability barrier.

Modification of ribozymes to reduce charge is just one approach to enhance the cellular uptake of these larger molecules. The random approach, however, is not advisable since ribozymes are structurally and functionally more complex than small drug molecules. The structural requirements necessary to maintain ribozyme catalytic activity are well understood by those in the art. These requirements are taken into consideration when designing modifications to enhance cellular delivery. The modifications are also designed to reduce susceptibility to nuclease degradation. Both of these characteristics should greatly improve the efficacy of the ribozyme. Cellular uptake can be increased by several orders of magnitude without having to alter the phosphodiester linkages necessary for ribozyme cleavage activity.

Chemical modifications of the phosphate backbone will reduce the negative charge allowing free diffusion across the membrane. This principle has been successfully demonstrated for antisense DNA technology. The similarities in chemical composition between DNA and RNA

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make this a feasible approach. In the body, maintenance
of an external concentration will be necessary to drive
the diffusion of the modified ribozyme into the cells of
the tissue.   Administration routes which allow the
5   diseased tissue to be exposed to a transient high
concentration of the drug, which is slowly dissipated by
systemic   adsorption   are   preferred.   Intravenous
administration with a drug carrier designed to increase
the circulation half-life of the ribozyme can be used.
10   The size and composition of the drug carrier restricts
rapid clearance from the blood stream. The carrier, made
to accumulate at the site of infection, can protect the
ribozyme from degradative processes.

Drug delivery vehicles are effective for both
15   systemic and topical administration. They can be designed
to serve as a slow release reservoir, or to deliver their
contents directly to the target cell. An advantage of
using direct delivery drug vehicles is that multiple
molecules are delivered per uptake.   Such vehicles have
20   been shown to increase the circulation half-life of drugs
which would otherwise be rapidly cleared from the blood
stream.   Some examples of such specialized drug delivery
vehicles which fall into this category are liposomes,
hydrogels, cyclodextrins, biodegradable nanocapsules, and
25   bioadhesive microspheres.

From   this   category   of   delivery   systems,
liposomes are preferred. Liposomes increase intracellular
stability,   increase   uptake   efficiency   and   improve
biological activity.

30   Liposomes are hollow spherical vesicles composed
of lipids arranged in a similar fashion as those lipids
which make up the cell membrane. They have an internal
aqueous space for entrapping water soluble compounds and
range in size from 0.05 to several microns in diameter.
35   Several studies have shown that liposomes can deliver RNA
to cells and that the RNA remains biologically active.

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For example, a liposome delivery vehicle originally designed as a research tool, Lipofectin, has been shown to deliver intact mRNA molecules to cells yielding production of the corresponding protein.

5.   Liposomes offer several advantages: They are non-toxic and biodegradable in composition; they display long circulation half-lives; and recognition molecules can be readily attached to their surface for targeting to tissues. Finally, cost effective manufacture of liposome-

10 based pharmaceuticals, either in a liquid suspension or lyophilized product, has demonstrated the viability of this technology as an acceptable drug delivery system.

Other controlled release drug delivery systems, such as nonoparticles and hydrogels may be potential

15 delivery vehicles for a ribozyme. These carriers have been developed for chemotherapeutic agents and protein-based pharmaceuticals, and consequently, can be adapted for ribozyme delivery.

Topical administration of ribozymes is

20 advantageous since it allows localized concentration at the site of administration with minimal systemic adsorption. This simplifies the delivery strategy of the ribozyme to the disease site and reduces the extent of toxicological characterization. Furthermore, the amount

25 of material to be applied is far less than that required for other administration routes. Effective delivery requires the ribozyme to diffuse into the infected cells. Chemical modification of the ribozyme to neutralize negative charge may be all that is required for

30 penetration. However, in the event that charge neutralization is insufficient, the modified ribozyme can be co-formulated with permeability enhancers, such as Azone or oleic acid, in a liposome. The liposomes can either represent a slow release presentation vehicle in

35 which the modified ribozyme and permeability enhancer transfer from the liposome into the infected cell, or the liposome phospholipids can participate directly with the

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modified ribozyme and permeability enhancer in facilitating cellular delivery. In some cases, both the ribozyme and permeability enhancer can be formulated into a suppository formulation for slow release.

5   Ribozymes may also be systemically administered. Systemic absorption refers to the accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes which lead to systemic absorption include: intravenous, subcutaneous,
10 intraperitoneal, intranasal, intrathecal and ophthalmic. Each of these administration routes expose the ribozyme to an accessible diseased tissue. Subcutaneous administration drains into a localized lymph node which proceeds through the lymphatic network into the
15 circulation. The rate of entry into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier localizes the ribozyme at the lymph node. The ribozyme can be modified to diffuse into the cell, or the liposome can directly
20 participate in the delivery of either the unmodified or modified ribozyme to the cell.

A liposome formulation which can deliver ribozymes to lymphocytes and macrophages is also useful for the initial site of influenza virus replication is in
25 tissues of the nasopharynx and respiratory system. Coating of lymphocytes with liposomes containing ribozymes will target the ribozymes to infected cells expressing viral surface antigens. Whole blood studies show that the formulation is taken up by 90% of the lymphocytes after 8
30 hours at 37°C. Preliminary biodistribution and pharmacokinetic studies yielded 70% of the injected dose/gm of tissue in the spleen after one hour following intravenous administration.

Intraperitoneal administration also leads to
35 entry into the circulation, with once again, the molecular weight or size controlling the rate of entry.

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Liposomes injected intravenously show accumulation in the liver, lung and spleen. The composition and size can be adjusted so that this accumulation represents 30% to 40% of the injected dose.

5 The rest is left to circulate in the blood stream for up to 24 hours.

The chosen method of delivery should result in cytoplasmic accumulation and molecules should have some nuclease-resistance for optimal dosing. Nuclear delivery

10 may be used but is less preferable. Most preferred delivery methods include liposomes (10-400 nm), hydrogels, controlled-release polymers, microinjection or electroporation (for *ex vivo* treatments) and other pharmaceutically applicable vehicles. The dosage will

15 depend upon the disease indication and the route of administration but should be between 100-200 mg/kg of body weight/day. The duration of treatment will extend through the course of the disease symptoms, usually at least 14-16 days and possibly continuously. Multiple daily doses are

20 anticipated for topical applications, ocular applications and vaginal applications. The number of doses will depend upon disease delivery vehicle and efficacy data from clinical trials.

Establishment of therapeutic levels of ribozyme

25 within the cell is dependent upon the rate of uptake and degradation. Decreasing the degree of degradation will prolong the intracellular half-life of the ribozyme. Thus, chemically modified ribozymes, e.g., with modification of the phosphate backbone, or capping of the

30 5' and 3' ends of the ribozyme with nucleotide analogues may require different dosaging. Descriptions of useful systems are provided in the art cited above, all of which is hereby incorporated by reference herein.
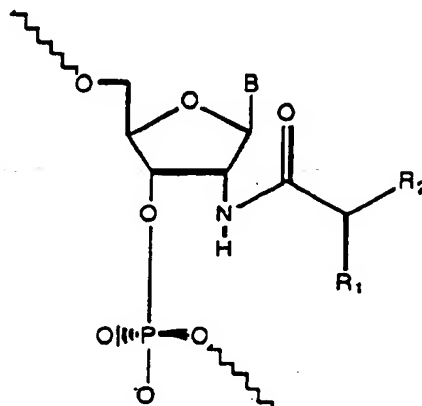
The claimed ribozymes are also useful as

35 diagnostic tools to specifically or non-specifically detect the presence of a target RNA in a sample. That is, the target RNA, if present in the sample, will be

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specifically cleaved by the ribozyme, and thus can be readily and specifically detected as smaller RNA species. The presence of such smaller RNA species is indicative of the presence of the target RNA in the sample.

5   Other embodiments are within the following claims.

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Claims

1.　Oligonucleotide comprising a nucleotide base having the formula:

wherein B is a nucleotide base or hydrogen; R_1 and R_2
5　independently is selected from the group consisting of
hydrogen, an alkyl group containing between 2 and 10
carbon atoms inclusive, an amine, an amino acid, and a
peptide containing between 2 and 5 amino acids inclusive;
and the zigzag lines are independently hydrogen or a bond.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 06556 (GILEAD SCIENCES, INC.) 16 May 1991 see the whole document, but especially page 17, example 1 ---	1
X	WO,A,88 00201 (CALIFORNIA INSTITUTE OF TECHNOLOGY) 14 January 1988 see the whole document, but especially page 66; page 68, lines 1-19; claim 1 --- -/--	1

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 1995	0 2. 03. 95

Name and mailing address of the ISA	Authorized officer
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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF CARBOHYDRATES NUCLEOSIDES AND NUCLEOTIDES, vol.7, no.5, 1980 pages 297 - 313 S.CHLADEK ET AL. 'Aminoacyl Derivatives of Nucleosides, Nucleotides and Polynucleotides. XXXII. Synthesis of Aminoacyldinucleoside Phosphates Derived from 2'- and 3'-Aminodeoxyadenosine.' see the whole document -----	1

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9106556	16-05-91	AU-A-	6715790	31-05-91
		EP-A-	0497875	12-08-92
		JP-T-	5504552	15-07-93
WO-A-8800201	14-01-88	US-A-	4849513	18-07-89
		DE-D-	3750080	21-07-94
		DE-T-	3750080	22-09-94
		EP-A-	0270651	15-06-88
		JP-T-	1501149	20-04-89